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Melanoma, Renal Thyroid Tumor and Reticulo-endothelial Hyperplasia in a Non-hybrid Platyfish[1]

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(Plates I & II; Text-figure 1)

MELANOMAS consistently occur in certain platyfish-swordtail and other interspecific hybrid combinations among fishes of the genus *Xiphophorus*. Tumors of the thyroid gland develop in certain species and strains of *Xiphophorus* when they are maintained in iodine-poor water. Genetic factors are important in the etiology of these atypical growths of pigment and thyroid cells (Gordon, 1958; Atz, 1959; Baker, 1958a; Mac Intyre, 1960).

The discovery, in our laboratory, of a *non-hybrid* platyfish with a melanoma evoked considerable interest, and when this specimen was also found to exhibit tumorous thyroidal growths in both the pharyngeal region and kidney, an abnormal pituitary gland and hyperplasia of reticulo-endothelial cells, the value of studying it in detail became even more apparent. During the study large pigmented masses, interpreted as melanin-laden macrophages, were found in the kidney—a phenomenon not known to have been previously described.

In these fishes, melanosis necessarily precedes melanoma. One melanotic specimen of the same species was found and is described.

Description of Melanomatous Fish with Thyroid Tumor

History and Gross Anatomy.—The specimen was an adult male spike-tailed platyfish, *Xiphophorus variatus xiphidium,* from the sixth laboratory-reared generation of fish collected in 1939 from the Rio Purificacion, Tamaulipas, Mexico (strain 53). The identification was verified by Dr. Donn E. Rosen of the American Museum of Natural History. The fish measured 28 mm. in standard length and 12 mm. in depth. It carried the dominant sex-linked gene, *Sp,* for macromelanophore spotting on the body. In this specimen much of the body was intensely black. Externally a swelling, which was caused by the melanoma, was visible on the right side underneath the dorsal fin (Text-fig. 1). The extent of the swelling was approximately $8 \times 6\frac{1}{2} \times 2$ mm. Its surface was smooth. The thyroid tumor was not visible externally.

The specimen was fixed in Bouin's fluid, decalcified in formic acid, embedded in paraffin and sectioned at 3, 7 and 10 microns; staining was with Harris's hematoxylin and eosin, Masson's trichrome stain or Heidenhain's iron hematoxylin. Before staining, the melanin in some sections was bleached with hydrogen peroxide or with potassium permanganate and oxalic acid.

Microscopic Appearance of the Melanoma.—The melanoma was essentially similar in microscopic appearance to those previously described in hybrids between *Xiphophorus maculatus* and *X. hellerii* by Reed & Gordon (1931), Gordon & Smith (1938), and others. It has caused extensive destruction of the body musculature (Plate I, Fig. 2). The dermis was infiltrated with tumor cells, and it contained melanin-laden macrophages. The epidermis was considerably thickened in some regions and also contained melanin-laden macrophages (Fig. 3). The scales were in abnormal positions.

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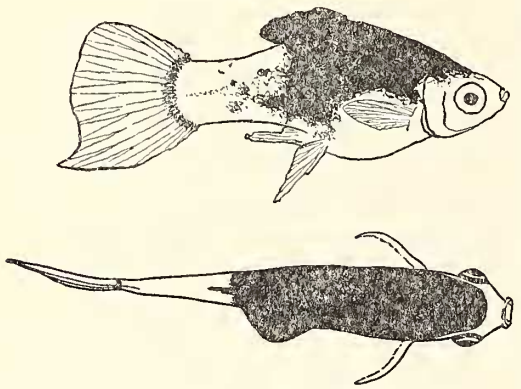
The predominant type of cell in the melanoma was the lightly pigmented melanocyte. There were scattered melanin-laden cells — macrophages and melanophores.[3] In areas adjacent to muscle cells, the melanocytes were arranged in whorls, and their nuclei were spindle-shaped and small, generally 2½ × 5 microns (Fig. 4). Elsewhere the nuclei of the melanocytes were round, oval or irregular and generally somewhat larger; the size varied but the majority were under 5 microns in diameter (Fig. 5). The cell outlines of the melanocytes were indistinct. The nuclei contained one or more prominent nucleoli. Mitotic figures were rare. A number of nuclei showed median or budlike constrictions, possibly indicating amitosis. Some giant nuclei were observed (Fig. 6).



TEXT-FIG. 1. Lateral and dorsal views of a male *Xiphophorus variatus xiphidium* with a melanoma.

Microscopic Appearance of the Thyroid Tumor.—The pharyngeal thyroid was markedly hyperplastic and extended into the bases of the gills (Plate II, Figs. 10, 11). This hyperplasia was chiefly made up of small to large follicles, all filled with dense, acidophilic colloid. The colloid was not vacuolated and varied from a very dense homogeneous condition in the small follicles to a course granular state in the large ones. Some afollicular cells occupied interstices between the follicles. Thyroid tissue was present in small amounts in the chorioid gland of the eye and was either follicular with granular colloid or afollicular. In the kidneys, thyroid tissue was represented by many large, colloid-filled follicles, similar to those in the pharynx, and by swollen cystic follicles almost or completely devoid of colloid (Figs. 12, 13). No thyroid tissue was seen elsewhere in the body. The renal thyroid was different from the thyroid tumors in the kidneys of *Xiphophorus maculatus* described by Baker *et al.* (1955) in that the normal kidney tissues were less disturbed by the proliferation of thyroid tissue than they were in *X. maculatus* during the late stages of tumor growth and that large amounts of colloid were present throughout both the pharyngeal and renal thyroid masses.

Hyperplasia of the Pituitary Gland.—Although the pituitary gland of this fish was not sectioned or stained in a manner suitable for the demonstration of cell types, it was at once apparent that the gland was abnormal (Plate II, Fig. 14). In the anterior part of the gland, the vertical cross-section was much elongated; the elongation was made up of a homogeneous mass of light-staining basophils. Eosinophils were not delineated by the staining methods employed. Normally, at that cross-sectional level, the pituitary is round and chiefly composed of darkly staining basophils; the paler cells appear in small numbers along the ventral border and increase somewhat in the posterior direction, as the neural portion of the hypophysis appears. In the tumorous fish, the anterior basophilic overgrowth also continued along the entire length of the pituitary, making the entire organ misshapen.

Hypertrophy of the paler basophilic elements of the pituitary gland has been found in *X. maculatus* with goitrous thyroids or with thyroid tissue regeneration following radioiodine treatment (Baker-Cohen, unpublished data). In such fish, however, the proliferation of pale basophils did not assume the proportions seen in the tumorous *X. v. xiphidium.*

Hyperplasia of the Reticulo-endothelial System.—In the kidney there were numerous areas of cells heavily laden with melanin (Plate II, Figs. 12, 13). These cells were spherical and from 5 to 10 microns in diameter. Their nuclei, which were visible only in bleached sections, were compressed at the periphery of the cells because of the large amount of melanin. The nuclei were oval or irregular in shape, generally 1 to 3 microns long and ½ to 1½ microns wide (Figs. 8, 9). In the anterior part of the melanoma there was a distinct area that consisted of a large mass of melanin-laden cells similar to the ones described above. This appeared to be a distinct growth and not part of the melanoma proper. This mass was ventral to the vertebral column, surrounding the urinary ducts and dorsal to the air bladder, which it compressed (Plate I, Fig. 7). Posteriorly this mass was reduced to a few cells ventral to the vertebral column (Fig. 1). A few such cells were found in

[3]In lower vertebrates, the melanocyte, which is the melanin-synthesizing cell common to all classes of vertebrates, differentiates into a pigment-effector cell called the melanophore; the melanophore is not a pigment-carrying macrophage (Gordon, 1953).

the liver, surrounding the brain, and in blood vessels within the brain. These cells appear to be macrophages, present in hyperplastic proportions (see Discussion).

Closely associated with the melanin-laden macrophages in the kidney were many nodular areas containing pale cells with indistinct outlines and round nuclei (2½ to 5 microns in diameter). Scattered macrophages were found in many of these nodules. We interpret these pale cells as primitive reticular cells, present in hyperplastic proportions. Some of these cells were also found associated with the area of melanin-laden cells in the anterior part of the melanoma. Extensive nodules of these cells were found among the pharyngeal thyroid follicles (Fig. 11). In the pharyngeal area, macrophages were rarely found in these nodules.

Description of Melanotic Fish

History and Gross Anatomy.—This specimen also was a laboratory-reared, adult male *X. v. xiphidium.* It was identified by Dr. Donn E. Rosen. The fish measured 27 mm. in standard length and 11 mm. in depth. It carried the *Sp* gene; in this specimen the body was almost completely black (blacker than the body of the melanomatous fish). The specimen was fixed in formalin, decalcified in formic acid, embedded in paraffin and sectioned at 7 and 10 microns; staining was with Harris's hematoxylin and eosin.

Microscopic Appearance of the Melanosis.—In the posterior part of the body, melanophores were present not only in the dermis but had spread along the septae of the body musculature. Some lightly pigmented melanocytes had also spread among the muscles, and in the left dorsal side melanocytes had multiplied sufficiently to compress some of the muscles. There was slight destruction of the musculature. The epidermis in this area was thickened. It is not possible to say whether, if the fish had remained alive, this melanosis would have developed into a melanoma.

Microscopic Appearance of the Thyroid Tissue and Pituitary Gland.—The pharyngeal thyroid appeared normal. It consisted of a number of follicles, 20 to 175 microns in diameter, none containing colloid (probably an artifact, resulting from formalin fixation). The follicular cells were squamous to cuboidal. No thyroid tissue was observed in the kidney. The kidney contained many pearl-like basophilic concretions, similar to those described by Berg *et al.* (1954) in regressing thyroid tumors that had been treated with potassium iodide.

The hypophysis of this fish, although not well preserved because of the slow formalin fixation, appeared to be normal. Its structure seemed to be the same as seen in *X. maculatus.*

Macrophage Hyperplasia.—There was a large mass of spherical melanin-laden cells, similar to those described as macrophages in the melanomatous *X. v. xiphidium,* ventral to the vertebral column, surrounding the urinary ducts and urinary bladder. Numerous masses of these cells were present in the kidney, and some were found in the lining of the air bladder. Some primitive reticular cells were also present in the kidney.

Discussion

Melanoma.—Thousands of wild and laboratory-reared poeciliid fish, including *Xiphophorus variatus xiphidium,* have been examined in this laboratory and elsewhere, but melanomas have been found only in interspecific hybrids, with the exception of the *Fu* (*fuliginosus*) domesticated strain of *X. maculatus* (Kosswig, 1938) and a few specimens of *X. montezumae cortezi* carrying the genetic factor for spotted caudal peduncle, *Sc* (unpublished data). The macromelanophore genes in platyfish require genetic buffering to control their growth, which is upset in hybrid combinations (Gordon, 1958; Atz, 1959). In the melanomatous *X. v. xiphidium,* which carried the *Sp* gene for macromelanophore spotting, the normal genetic constitution of the species was presumably altered, perhaps as a result of mutation or inbreeding.

Thyroid Tumor.—The discovery of extensive thyroidal proliferation in the kidneys of the melanomatous *X. v. xiphidium* adds another species to the growing roster of teleosts in which extra-pharyngeal thyroid tissue has been found. These have been summarized recently by Baker (1959) and Baker-Cohen (1959). In all cases to date, the fish exhibiting displaced thyroid tissue have had some history of a domesticated environment, sometimes with a known iodine deficiency, and all were freshwater species.

Both the pharyngeal and renal thyroidal growths in the *X. v. xiphidium,* like those described in a cherry barb, *Barbus titteya* (Baker, 1959), and unlike those reported in common platyfish, *X. maculatus* (Baker *et al.,* 1955), were extensively colloidal. They thereby more nearly resembled colloid goiter than adenoma, as described in man.

Thyroid tumors have rarely been found in this species. However, they may be more frequent than previously supposed if they are not visible externally, as was the present one. Thyroid tumors in poeciliid fish generally produce externally-visible swellings of the gill region or of the body.

Relation between the Melanoma and Thyroid Tumor.—Stolk (1959) recently reported that the incidence and development of melanomas was increased in thiouracil-fed (hypothyroid) platyfish-swordtail hybrids and reduced in thyroxine-fed (hyperthyroid) ones. Similar results were obtained by Meites (1958) for carcinogen-induced skin tumors in Swiss mice; his article gives references to the few and contradictory studies that have been made on the influences of the thyroid gland on tumor growth.

Endocrine secretions, particularly certain pituitary hormones, play an important role in pigment cell formation and maturation and in melanin synthesis in normal fish (Pickford, 1957; Chavin, 1959; Kosto *et al.,* 1959). Considering the extreme rarity of melanomas in *X. v. xiphidium,* it is conceivable that the development of the melanoma described here might have been influenced by the abnormal state of the fish's endocrine system, particularly in view of the unusual state of hyperplasia of the hypophyseal basophils.

Hyperplasia of the Reticulo-endothelial System.—Morphologically, the melanoma and melanosis described here are similar to those reported in platyfish-swordtail hybrids, with the exception of the large number of heavily melanin-laden cells in the kidney and ventral to the vertebral column. Metastases are very rare in fish melanomas. Breider (1938, 1953) described pigment deposits in the kidneys of melanomatous platyfish-swordtail hybrids. He considered them to be acellular pigment deposits but did not make any studies of bleached sections; the deposits shown in his figures appear very similar to the masses of melanin-laden cells found in our *X. v. xiphidium.* Mac Intyre (unpublished data) has found similar, though less extensive, masses in the kidneys of some melanomatous *X. maculatus* belonging to the *Fu* strain and in some melanomatous platyfish-swordtail hybrids. We do not believe that these represent metastases, but rather macrophages that have both multiplied to hyperplastic proportions and massed in these areas. Cell movement alone could not account for the large quantity of these cells.

What happens to melanin after it has been produced by the melanocyte is not fully known (Dalton & Felix, 1953). Much of it is transferred to macrophages. The recent findings of Speece *et al.* (1959) in human melanomas indicate that macrophages not only ingest dead cells but pick up melanin discharged into the intercellular fluid by active tumor cells. Observation of cut pigment cells in amphibians showed that some melanin-containing macrophages remained at the area of ingestion, while others migrated and entered the blood stream (Lehman, 1953). Melanin debris may not be immediately ingested by macrophages; it may be removed from tissue spaces by the lymphatics (Smith, 1931) and thus carried to lymph nodes or other areas of macrophage concentration. Ultimately, the melanin may be broken down intracellularly in macrophages, or, as Cowdry (1950) stated, "once formed, melanin is a very stable substance and may be excreted in the urine." In fish and amphibians, some melanin-containing macrophages are sloughed off through the epidermis (Goodrich & Hansen, 1931; Gordon & Lansing, 1943; Niu, 1959). Niu (1959) has suggested that in amphibians the pigment debris is generally carried by macrophages to the liver, where it is eliminated via the biliary system.

In teleost fishes, the kidney is the organ richest in lymphoid tissue (Bertin, 1958) and the principal hemopoietic organ (Grassé, 1958). In the kidney sinuses of normal poeciliid fish, large numbers of lymphocytes and erythrocytes and small numbers of primitive reticular cells and macrophages are found. Primitive reticular cells can differentiate into lymphocytes and macrophages (Walvig, 1958). Studies in the eel have shown that injected dye and bacteria are picked up chiefly by lymphoid cells in the kidney (Schmidt, 1959). Bertin (1958) stated that ink or carmine injected into a fish's body is eliminated in the urine, after several hours, across the renal lymphoid tissue.

In the two specimens of *X. v. xiphidium,* histological observations indicate that much of the enormous amount of melanin produced by the melanoma and melanosis was picked up by macrophages in the kidney, which were evidently not able to eliminate the melanin, by intracellular digestion or by transfer into the urine, at the rate that it was produced by the abnormally large number of melanocytes. In addition to this process, much of the melanin was phagocytized by macrophages in the immediate area, some of which remained there and others of which were sloughed off through the epidermis. That these are methods of melanin disposal in poeciliid fishes is confirmed by observations we have made in normally pigmented fish: *e.g.* small numbers of macrophages containing melanin are customarily found in the kidney and other lymphoid tissues.

The nodules of primitive reticular cells found in the kidney and in the pharyngeal thyroid tumor resemble cells that were found by Rasquin & Rosenbloom (1954) in *Astyanax* raised in the dark and described by them as reactive hyperplastic reticulo-endothelial cells. Baker (1958b)

found similar masses, associated with degenerating thyroid follicles, in *X. maculatus* whose renal thyroid tumors had been treated with potassium iodide.

Relations between the Reticulo-endothelial Hyperplasia and the Melanoma and Thyroid Tumor.—Several studies have been made in mammals of the relation between the reticulo-endothelial system and neoplastic diseases (*e.g.* Pelner, 1957; Old *et al.*, 1960). The latter found a slight increase of RES activity during the growth of certain spontaneous mouse tumors and suggested that this activation might represent response to some unique property of the tumor, stimulation by products of tumor growth, or response to associated necrosis, hemorrhage, or infection. In the fish with the melanoma and thyroid tumor, we conclude that there was a marked reactive hyperplasia of the reticulo-endothelial system, particularly of macrophages and primitive reticular cells, as a result of the presence of the tumors, for one or more of the above reasons. Reaction to excessive melanin production may have been a causative factor in the hyperplasia, or the melanin phagocytosis may have been a secondary phenomenon. The cells of this reactive hyperplasia were present in such great quantity that they themselves probably caused considerable damage through pressure on the air bladder and kidney tissue.

Summary

Melanomas consistently occur in certain platyfish-swordtail and other interspecific hybrid combinations of xiphophorin fishes. A melanoma in a non-hybrid xiphophorin, the spike-tailed platyfish, *Xiphophorus variatus xiphidium,* is described, in which the normal genetic mechanisms buffering pigment cell growth were presumably upset. The melanoma consisted mainly of lightly pigmented melanocytes and had destroyed much of the body musculature on the right side. This fish had an extensive thyroid tumor in the pharyngeal region and in the kidney, consisting of colloid-filled and swollen cystic follicles. In the pituitary gland, a hyperplasia of light-staining basophils was present.

In addition to these abnormal growths, there was a marked reactive hyperplasia of the reticulo-endothelial system, consisting of large masses of melanin-laden macrophages in the kidney and ventral to the vertebral column, and of nodules of primitive reticular cells in the kidney and in the pharyngeal thyroid tumor. The disposal of melanin and the reticulo-endothelial system in fish are discussed; it appears that much of the melanin produced by pigment cells is picked up by phagocytic cells in the lymphoid tissue of the kidney.

Another specimen of *X. v. xiphidium* is described, in which melanosis was present. Large masses of melanin-laden macrophages were also found in its kidney and ventral to the vertebral column.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Cross section of a melanomatous male *Xiphophorus variatus xiphidium* through the posterior part of the melanoma, which consists mainly of lightly-pigmented melanocytes. 13×.

Fig. 2. Infiltration of the musculature by melanocytes. 140×.

Fig. 3. Thickening of the epidermis. 260×.

Fig. 4. Bleached section in an area of muscle infiltration, showing whorls of melanocytes with small spindle-shaped nuclei. The large dark objects are muscles. 345×.

Fig. 5. Bleached section from a more central part of the tumor. The nuclei are larger and round or irregular in shape. 920×.

Fig. 6. A giant nucleus. 700×.

Fig. 7. Cross section through the anterior part of the melanoma. To the left are melanocytes which have invaded the musculature. A large hyperplastic mass of melanin-laden macrophages occupies part of the region where the air bladder is normally located. 70×.

Plate II

Fig. 8. Bleached section showing the edge of the melanin mass of Fig. 7. At the right are melanin-laden macrophages which, after bleaching, appear as empty shells. At the left are melanocytes. 450×.

Fig. 9. Bleached melanin-laden macrophages. The small, irregularly-shaped nuclei are compressed at the periphery of the cells. 945×.

Fig. 10. Thyroid tumor in the same fish. Pharyngeal region. 70×.

Fig. 11. Detailed view of pharyngeal region. At the right are thyroid follicles. At the left is a mass of hyperplastic primitive reticular cells. 375×.

Fig. 12. Thyroid tumor in the kidney showing large colloid-filled follicles and a large cyst at the upper right. The solid black areas are groups of hyperplastic macrophages, laden with melanin. 70×.

Fig. 13. Detailed view of melanin-laden macrophages and thyroid tissue in kidney. 375×.

Fig. 14. Hyperplasia of light-staining basophils in the pituitary gland of the same fish. 100×.